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U.S. DEPARTMENT OF ENERGY | Office of Science

LIFE SCIENCES DIVISION E-NEWSLETTER

August/September, 2009

In this issue:

DOE scientific focus area notes

Low Dose Radiation Research

Comprehensive Investigation of the Low Dose Radiation Responses in Tissues
Exposure to high doses of ionizing radiation is known to lead to a variety of health effects such as tissue pathology, chromosomal damage, life shortening and cancer. It is also known that tissues differ dramatically in their sensitivities to radiation-induced DNA damage, cell death and carcinogenesis. However, the tissue response and the health effects after exposure to low doses of ionizing radiation are largely unknown. Radiation-induced modulation of gene-expression has been observed among tissues and cell lines, but their significance for long-term consequences is poorly understood. It is clear however that that the tissue response at low dose is different from that at high dose.

As part of the DOE Low dose Scientific Focus Area (SFA), Life Sciences Division scientists **Andrew Wyrobek**, **Francesco Marchetti**, **Antoine Snijders** and others, have now conducted a comprehensive study aimed at elucidating the *in vivo* damage response in mouse tissues after whole body radiation exposures to identify and characterize the molecular mechanisms that provide differential sensitivity for initial genomic and chromosomal damage, for persistent genomic instability, senescence and cancer and how these responses are different between high and low dose radiation. These investigations are being carried out in tissues of both male and female mice from inbred strains that are known to differ in their radiation susceptibilities for lymphoma and mammary gland cancer. With the technical support of the Life Sciences Division High Troughput Analysis (HTA) group, a total of 930 genome-wide microarray experiments were conducted to survey gene expression levels in multiple tissues after exposure to low and high dose radiation. Both single and multiple exposure regimens were utilized. Surveyed tissues included: mammary gland, lymph node, thymus, bone marrow, blood, cerebellum, cortex, hippocampus, and testis. The majority of tissues were collected within 4-10 hrs after exposure to identify the early response to ionizing radiation, however, a subset of tissues were collected 1 month after exposure to identify persistent and late changes in gene expression.

In collaboration with the Low dose SFA bioninformatics group, initial analysis is focusing on the low dose radiation response of the mammary gland in BALB/c (sensitive to radiation induced mammary gland cancer) and C57BL/6 (resistant to radiation induced mammary gland cancer) mice to identify individual genes, gene interaction networks and molecular pathways associated with the low dose response and sensitivity to radiation induced mammary gland cancer. These results will be compared to the response of the mammary gland of animals exposed to high doses of ionizing radiation. This project is expected to provide sensitive and specific measures of the shape of the dose-response curve in the low-dose region. Ultimately, the current project is expected to provide molecular mechanisms associated with the low dose response, which underlie individuals' genetic susceptibility for radiation-induced tumorigenesis and other radiation-induced diseases. These mechanisms can then be integrated into a systems biology model for the assessment of risk associated with low dose exposures, facilitating DOE's regulatory decision making.
Francesco Marchetti/ Antoine Snijders, 9/09

GTL-Genomics

PCAP Study Published in PNAS

Bong-Gyoon Han, **David Ball**, **Dieter Typke**, **Ken Downing**, and **Robert Glaeser** of the Life Sciences Division, **Mark Biggin** of the Genomics Division, together with colleagues from the Genomics, Earth Sciences, and Physical Biosciences Divisions and the University of California, San Francisco, have published their study on multiprotein complexes in the September 11, 2009 on-line early edition of *Proceedings of the National Academy of Sciences*. The work was part of the Protein Complex Analysis Project (PCAP) funded by the DOE Genomics:GTL Program.

The major insight gained from this work is that the subunit compositions and quaternary structures of biochemically related multiprotein complexes varies from one microbe to another to a far greater extent than heretofore had been appreciated. Out of the 16 largest, most abundant complexes from *Desulfovibrio vulgaris* Hildenborough (*Dv*H) that were characterized in this proteomic survey, for example, only 2 (the 70s ribosome and GroEL) could have been accurately modeled on the basis of information that exists in current data bases.

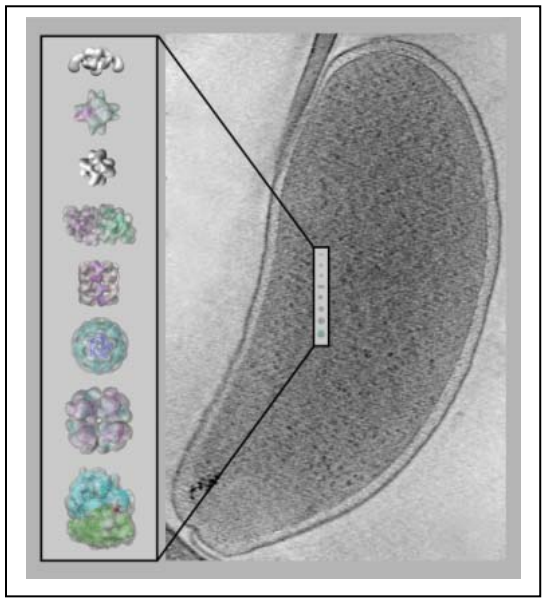


Image: Gallery of multiprotein complexes whose structures were determined by electron microscopy as part of the survey. These are shown on the left at a large enough magnification to appreciate that each type of complex is recognizable on the basis of its unique size and shape, and then again zoomed down to the size scale at which they actually exist within the magnified image of a parent cell of Desulfovibrio vulgaris Hildenborough.

The proteomic survey by the authors, which combined structural characterization by single-particle electron microscopy with tagless purification of multiprotein complexes, identification of constituent polypeptides by mass spectroscopy, and analysis of the data by bioinformatics tools, thus provides strong validation of one of the premises of DOE's GTL program, namely that it is necessary to develop high-throughput technologies for characterization and imaging of the particular versions of multiprotein complexes that exist within each microbe of interest. As pointed out in this paper, relying solely on previously determined quaternary structures for homologous proteins is unlikely to be sufficient to properly understand the enzyme kinetics of the particular protein complexes that are favored in *Dv*H, and it definitely would not provide sufficient information to locate most of the complexes within cryo-EM tomograms by template matching.

Han B- G, Dong M, Liu H, Camp L, Geller J, Singer M, Hazen TC, Choi M, Witkowska HE, Ball DA, Typke D, Downing KH, Shatsky M, Brenner SE, Chandonia JM, Biggin MD, Glaeser RM. Survey of large protein complexes in *D. vulgaris* reveals unexpected structural diversity. *Proceedings of the National Academy of Sciences USA*. Sept. 2009

Robert Glaeser, 9/09

Radiochemistry & Instrumentation

Collaboration with Engineering Division
Scientists in the Department of Radiotracer Development and Imaging Technology have been engaged in an OBER-funded collaboration with the Berkeley Lab Engineering Division to design a high performance ASIC (application specific integrated circuit) that will greatly enhance the readout of arrays of silicon photomultipliers (SiPMs), a recently developed type of photodetector that shows great promise for radiotracer imaging instruments. The final engineering design review for this custom integrated circuit was held on September 16, 2009 and no major issues were found. They are on schedule to submit the chip for fabrication in early November.
Bill Moses, 9/09

Scientific & divisional news

Gray Reappointed to Board of Scientific Advisors
Life Sciences **Joe Gray** has accepted his reappointment to the National Cancer Institute (NCI) Board of Scientific Advisors for another four years. Gray has served the Board since 2004. It consists of 35 members, including the Chair appointed by the NCI Director, from authorities knowledgeable in the fields of laboratory, clinical and biometric research, clinical cancer treatment, cancer etiology, and cancer prevention and control. Members serve for overlapping terms of up to five years. As necessary, subcommittees are established, such as the Ad Hoc Subcommittee for the "The Childhood Cancer Therapeutically Applicable Research to Generate Effective Treatments (TARGET)" Initiative, chaired by Gray.

The Board provides scientific advice to the Director and Deputy Director for Extramural Science, NCI, and the Director of each NCI Division on a wide variety of matters concerning scientific program policy, progress and future direction of NCI's extramural research programs. This includes the evaluation of NCI awarded grants, cooperative agreements and contracts and concept review of those activities which it considers meritorious and consistent with the Institute's programs. The advisory role of the Board is scientific and does not include deliberation on matters of public policy. As necessary, the Board and its subcommittees call upon special consultants, assemble ad hoc working groups, and convene conferences, workshops, or other activities.
Today at Berkeley Lab/CG, 8/3/09

Auer is Man of the Hour for Mapping the Cell Wall
Since all of the action of breaking down lignin and other plant constituents to fermentable sugars will take place in the cell walls of second-generation biofuel feedstocks, it is probably a good idea to know the exact make-up of a cell wall. That complex challenge is being accepted by electron microscopist **Manfred Auer** and his team of imaging


Manfred Auer (above right)

specialists at Berkeley Lab, Life Sciences Division, and UC Berkeley. "We need a realistic model of a cell wall to understand the 'building principles' that nature has come up with," said Auer. More> http://www.energybiosciencesinstitute.org/index.php?option=com_content&task=view&id=244&Itemid=1
Today at Berkeley Lab, 8/18/09

Tests Begin on Drugs That May Slow Aging

[*New York Times*] Excitement among researchers on aging has picked up in the last few years with the apparent convergence of two lines of inquiry: single gene changes and the diet known as caloric restriction. However, two experts on aging, including **Judith Campisi** of the Life Sciences Division, argued recently in *Nature* that the whole phenomenon of caloric restriction may be a misleading result unwittingly produced in laboratory mice. The mice are selected for quick breeding and fed on rich diets. A low-calorie diet could be much closer to the diet that mice are adapted to in the wild, and therefore it could extend life simply because it is much healthier for them. More > http://www.nytimes.com/2009/08/18/science/18aging.html?_r=1
Today at Berkeley Lab, 8/19/09

Life Sciences Researchers Host CSEE Summer Interns

Berkeley Lab's Center for Science and Engineering Education (CSEE) hosted a poster session on August 11, 2009. Featuring the work of 110 undergrads, this event is one of the rare opportunities to bring together examples of the breadth of research conducted at the Laboratory through the experiences of the teachers and students participating in CSEE mentored internship programs this summer. 26 students participated in the many CSEE Programs at Berkeley Lab: ACTS, BLIPS, BLUFF, BLUR, CCI, FaST, PST/CalTeach, PST/STAR, SULI (http://csee.lbl.gov/Programs/index.html) under the mentorship of Life Sciences Division researchers.

One of the interns participating in the DOE sponsored Pre-Service Teachers (PST) program, which places students in paid internships in Science, Math, and Technology to conduct research at Berkeley Lab, was **Jenny Hung**. While conducting research, PST interns were also required to concurrently take a weekly class, entitled "Integrating Research in Science Classrooms K-12," at the University of California, Berkeley. Ending in mid-August, the ten weeks summer program gave PST interns like Hung **"**the unique opportunity to expound upon her scientific understanding and to maximize her potential as a life-long educator". Hung selected **Joe Gray** as her mentor "after reading about the distinguished work he has conducted in breast cancer," she wrote. Furthermore, after 4 years of conducting breast cancer research in public health at the University of California, San Diego (UCSD), Hung was interested in exploring the same research in a laboratory setting. Since taking her first biology course in high school, she has had a strong appreciation and inclination towards the sciences, especially for biological sciences.

From working with the researchers in the Gray laboratory, particularly with scientist **James Korkola**, who worked with Hung on her current research project, Hung believes "she has received the highest quality education that has aided in her research abilities. Moreover, this experience has helped me reaffirm my direction in medicine and has shown me that in order to become a great physician I need to be a great

researcher and teacher as well". Currently, Hung is continuing her research endeavors with the Gray laboratory through the Science Undergraduate Laboratory Internship (SULI) program and plans on pursuing a teaching credential within the next year.
Today at Berkeley Lab/ CG, 8/6/09

Gray Speaks at Summer Lecture Series
The 2009 Berkeley Lab summer lecture series included a talk by **Joe Gray** who explored personalized cancer treatment on August 4. Gray talked on "Genome Science and Personalized Cancer Treatment": results from the Human Genome Project are enabling scientists to understand how individual cancers form and progress. This information, when combined with newly developed drugs, can optimize the treatment of individual cancers. Gray focused on this approach, its promise, and its current roadblocks – particularly with regard to breast cancer.

The series underscored the Lab's leading role in cutting-edge research, also including talks by Curtis Oldenburg on geologic carbon sequestration, Alexie Leauthaud 's and Reiko Nakajima's talk about gravitational lensing, and the talk by Bob Schoenlein on ultrafast science: using lasers and X-rays to reveal the motion of atoms and electrons.
Today at Berkeley Lab/CG, 9/2/09

Tainer and Gray Co-chair Transient Molecular Complexes Workshop
John Tainer and **Joe Gray** co-chaired a workshop on new approaches to research into Transient Molecular Complexes (TMC). The workshop, held on August 24-25, 2009 in San Francisco, was organized by the Division of Cancer Biology of the National Cancer Institute. The goal of the workshop was to discuss current opportunities and future challenges for understanding the role of TMC in the development and progression of cancer.

The working definition of transient molecular complexes is those macromolecular assemblies that are formed in response to specific signaling events such as chemical modifications or physical environment and exist long enough to affect an observable outcome. Notable examples are found in transcription complexes, DNA repair complexes, and cell signaling complexes. For the most part the structure and activity of critical core complexes are well defined but it's clear that these core complexes transiently interact with a host of factors (often shared) that are critical to cell viability. Relatively subtle shifts in this interactome can lead to dramatic changes that result in the development and progression of cancer. A detailed map of the interactome would have a major impact on the ability to understand, model, and treat a host of diseases. It's clear that developing this map will require the coordinated development of methods for identifying, quantifying, visualizing, and modeling transient interactions.

The program included both talks and poster presentations so that the scientifically diverse group of attendees could better understand each other's work.
CG, 8/09

Budinger Speaks at Military Health Research Forum

Thomas Budinger gave an invited lecture, "Early Detection of Mild Traumatic Brain Injury by Battlefield Imaging," at the Military Health Research Forum in Kansas City, Missouri, August 31-September 3, 2009. Budinger spoke about battlefield causalities having three major problems that are not adequately handled in the care of an injured warfighter. First, for many injuries it is not known if and when to replace blood loss, as blood pressure and pulse are kept in normal ranges by the sympathetic body responses, and not until compensatory mechanisms fail does the system collapse. A way to measure the severity of low oxygenation is by spectroscopic measures of deep muscle oxygenation rather than by the usual superficial measurements as is done with the pulse oximeter. Methods to improve on the portable systems invented by others were presented. The second major problem is detection of occult brain trauma such that the warfighter is not sent back to duty without further evaluation and treatment of brain hemorrhage or early edema before it is too late. Methods for detecting brain injury by portable systems in the battlefield were presented. The third innovation discussed was Berkeley Lab's effort to perfect a wireless controller using tongue touches to a keyboard in the roof of the mouth. This device is to be used by individuals that have lost capabilities to control computers and other devices.

The Military Health Research Forum is a scientific conference for presenting research studies funded by the Department of Defense's Congressionally Directed Medical Research Programs (CDMRP). The conference is jointly hosted by the CDMRP's Peer Reviewed Medical Research Program, Gulf War Illness Research Program, and Psychological Health and Traumatic Brain Injury Research Program.
Thomas Budinger, 9/09

Life Sciences Researchers Present Work at International Radiology and Nuclear Medicine Meeting

The Life Sciences Division was well represented at the 10th International Meeting on Fully Three-Dimensional Image Reconstruction in Radiology and Nuclear Medicine, September 5-10, 2009, in Beijing, China. Several oral presentations were given:

Gengsheng Zeng and **Grant Gullberg** presented on “Exact iterative reconstruction for the interior problem”; **Bryan Reutter**, **Rostyslav Boutchko**, **Ronald Huesman**, **Anne Sauve** and Grant Gullberg on “Penalized least-squares dynamic pinhole SPECT image reconstruction using a smooth 4-D image prior and multiresolution spatiotemporal B-splines”; and Qiu Huang, Tsutomu Zeniya, Hiroyuki Kudo, Hidehiro Iida and Grant Gullberg on ”Interior SPECT reconstruction problem with tiny a priori knowledge-an application for high resolution pinhole brain imaging.”

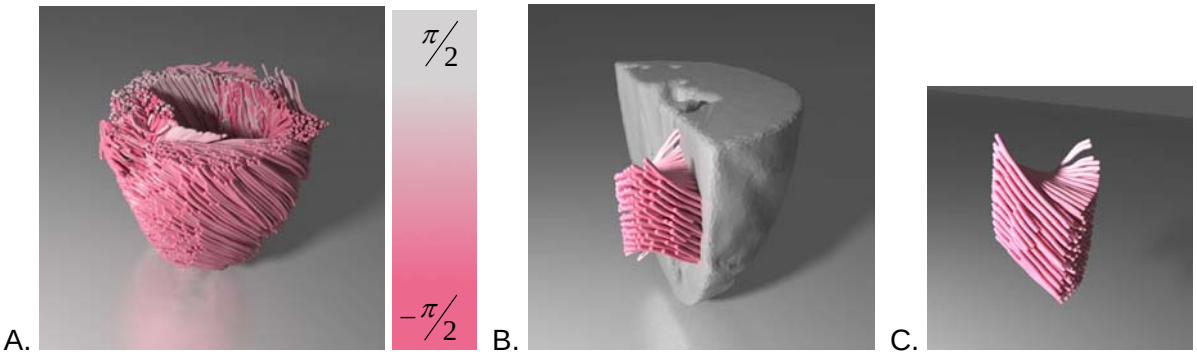
Posters were also presented: “Practical aspects of tomographic reconstruction on tetrahedral meshes” by Rostyslav Boutchko, Arkadiusz Sitek and Grant Gullberg; “Fully 3D SPECT attenuation and scatter correction using Monte Carlo generated system matrices” by Anne Sauve, Bryan Reutter and Grant Gullberg.
Stephen Derenzo, 9/09

Life Sciences Researchers Present Work at 2009 World Molecular Imaging Congress

Several members of the Life Sciences Division presented their work at the 2009 World Molecular Imaging Congress on September 23–26, 2009, in Montréal, Canada. **Bryan Reutter**, **Ronald Huesman**, **Kathleen Brennan**, **Rostyslav Boutchko**, **Stephen Hanrahan** and **Grant Gullberg** presented a poster on **"**Longitudinal evaluation of metabolic rates for glucose and fatty acid in normal and spontaneously hypertensive rat hearts with dynamic microPET and microSPECT imaging."

Also, Grant Gullberg presented research by himself and colleagues **James O'Neil,** Mustafa Janabi, Kathleen Brennan, Hilla Wahnishe, Youngho Seo and **Henry VanBrocklin** on "Cardiac perfusion as a function of cardiac hypertrophy using microPET/CT imaging of F-18-fluorodihydrorotenol". Gullberg spoke about fiber tracking of DTMRI data of an excised rat heart acquired on a 7.1T small animal magnet that was performed to create 3D visualizations of the fiber structure of the left ventricle. This is part of a project to follow changes in cardiac function and structure with the progression of cardiac hypertrophy in the spontaneous hypertensive rat. It is anticipated that following changes in fiber structure over a two-year period knowledge will be obtained of how the heart remodels its fiber structure response to hypertension.

The DTMRI data were acquired at the University of Utah by Dr. Edward Hsu (Associate Professor of Bioengineering and Director of the Small Animal Imaging Facility), and the fiber tracking of the DTMRI data and visualization was performed by Damien Rohmer, a former Berkeley Lab employee and now a Ph.D. student at the Grenoble Institute of Technology, Grenoble, France. The heart was excised from one of the SHR rats that is being studied with microPET and is part of the research study funded by Gullberg's NIH R01 grant, "Molecular Imaging of Cardiac Hypertrophy Using microPET and Pinhole SPECT."



Images: Visualization of the fiber structure of the left ventricle in the spontaneous hypertensive rat (SHR): A. The visualization was created by using a cylindrical mesh throughout the entire volume of the left ventricle. Each cylindrical element was determined starting with a seed point and tracking the principal component of the 3D distribution of the diffusion tensor obtained from DTMRI. B. & C. Left-handed to right-handed rotation of the fibers going from the epicardium to endocardium can be seen. The fibers are color coded corresponding to their angle at a specific position in space.

Stephen Derenzo, 9/09

Life Sciences Support 'Women in Science' Meeting

Laura Heiser

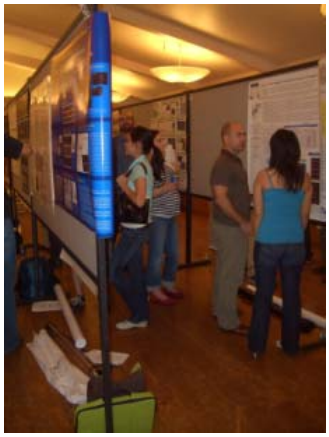
Life Sciences Division Scientist **Laura Heiser** presented her work at the bi-monthly chapter meeting of the East Bay Association for Women In Science (EbAWIS) on September 24, 2009 at Novartis in Emeryville. She discussed "The Road to Personalized Medicine: Identifying Therapeutic Agents Effective Against Subpopulations of Breast Cancers." Heiser explained that Breast cancer is an extremely heterogeneous disease in which individuals show a varied array of changes in the genetic makeup of their tumors. These genetic changes can be used to group breast cancers into different classes. Given this heterogeneity, a critical issue in treating patients is identifying the therapeutic interventions that will be most beneficial. In order to determine the types of breast cancers that may best respond to particular therapies, Heiser and colleagues treated about 50 breast cancer cell lines with about 70 drugs and measured their drug sensitivities. With this approach, they identified several therapeutics that show a class-specific bias, indicating that they would be effective for treatment of particular subpopulations of breast cancer patients.

Mara Jeffress

The event was organized by EbAWIS, the local chapter of the national Association of Women in Sciences (AWIS) advocacy organization championing the interests of women in science, technology, engineering, and mathematics across all disciplines and employment sectors. By breaking down barriers and creating opportunities, AWIS strives to ensure that women in these fields can achieve their full potential. The Association's current President is **Mara Jeffress**, who is a researcher in the Life Sciences Division as well. More> http://www.ebawis.org/
Today at Berkeley Lab/CG, 9/17/09

Life Sciences Division Holds Annual Scientific Retreat

The Life Sciences Division held her Annual Scientific Retreat on September 11, 2009 at the Joaquin Miller Community Center in Oakland, CA. The program committee, consisting of **Priscilla Cooper** and **Andrew Wyrobek**, Cancer & DNA Damage Responses Department Head and Deputy, respectively, and **Kelly Trego**, Scientist in that Department and representing the Postdoc Society, created an exciting program that attracted nearly 130 attendees and lead to the success of the retreat.



A fundamental element of the program was the participation by postdocs and scientists in a poster session, as well as in several 15 minute platform presentations. All postdocs and scientists were invited to submit a poster abstract; More than 55 researchers took advantage of the opportunity to share their work with the Division by presenting a poster. Time in the program was also reserved to honor two colleagues who passed away earlier in the year: **Aloke Chatterjee**'s work was presented by colleague **Amy Kronenberg** and Dr. Cardiff of the University of California, Davis presented the work of **Shyamala Harris**.

The abstracts selected for talks featured both postdocs and scientists were most lucid and compelling, and were chosen to reflect the diversity of research within the division. Most speakers were selected based on submitted abstracts but the program also featured invited speakers from the Division and a guest speaker of Berkeley Lab's Molecular Foundry, Bruce Cohen. **Joe Gray** concluded the program of talks by presenting a brief Division perspective. To allow a timely start of the poster session and reception he cut his scientific presentation short. Instead, he will be presenting his research at the Life Sciences and Genomics Divisions Seminar of February 23, 2010.
CG, 9/09

DNDO Grant Reviewed
Stephen Derenzo's grant from the Department of Homeland Security's Domestic Nuclear Detection Office (DNDO), "High-Throughput Discovery of Scintillation Materials," passed its annual review on September 29, 2009. Dr. Alan Janos, a program manager from DNDO, visited Berkeley Lab for a tour of the new crystal-growing facility in Building 64, presentations of past accomplishments and future tasks and plans, and a discussion of producing new scintillators in quantity.
Stephen Derenzo, 9/09

Awards

Conboy Gets $1.4 Million from NIH to Study RNA Splicing


John Conboy

John Conboy of the Life Sciences Division is receiving $1.4 million over two years for research into basic mechanisms that control gene expression during production of red blood cells in the bone marrow. The funds were made available by the American Recovery and Reinvestment Act and were awarded by the National Institutes of Health. Conboy's studies focus on understanding a molecular switch in RNA splicing that occurs in red cell precursors. Understanding RNA splicing is important because proper splicing is essential for normal development. Conversely, aberrant splicing is a common cause of genetic disease and a factor in many human cancers.
Today at Berkeley Lab, 9/25/09

Life Sciences Division Receives $1.3 million to Study Huntington's Disease
Cynthia McMurray of the Life Sciences Division is receiving $1.3 million over two years (funded for the first year at $611,104) for research into mechanisms that prevent or delay the onset and progression of Huntington's disease (HD). HD is a neurodegenerative disease that is expected to affect 200,000 Americans in the next decade, yet no effective long-term approaches to therapy are currently available. This project will build on and explore recent discoveries that DNA oxidative damage causes the somatic expansion in HD that is observed with age and governs onset that begins around mid-life, and that loss of expansion is accompanied by an unforeseen amelioration or delay of pathophysiology.
Today at Berkeley Lab, 9/30/09


Cynthia McMurray

Cancer Study Receives Supplemental Award

The study "System-Based Predictions of Responses to Cancer Therapy," co-lead by Life Sciences Joe Gray and Frank McCormick of the University of California, San Francisco, has been awarded an Administrative Supplement from the NIH National Cancer Institute of $756,483 total cost and a 6-month extension for the parent grant for $1,317,537, ending August 31, 2010.

The goal of this study is to promote the analysis of cancer as a complex biological system by supporting the development of reliably predictive in silico or computational models of cancer initiation and progression that can ultimately lead to the development of improved cancer interventions. It integrates experimental and computational approaches towards the understanding of cancer biology. The initiative encourages the emergence of integrative cancer biology as a distinct field. The program aims to develop and experimentally validate computational models of EGFR-family signaling in breast cancer that will predict individual responses to therapeutic agents that target this pathway. The program is one of six NIH National Cancer Institute (NCI) funded ICBP Centers in the United States.

The supplement will allow the researchers to accelerate research activities, hiring additional staff, and to obtain a more in depth analysis within the scope of the parent grant, a study which started in 2004 and continues to probe the Raf-Erk-Mek signaling network utilizing pathway modeling to assess pathway function and identify molecular markers of resistance (Project 1), elucidating cellular and molecular responses to targeted therapy (Project 2), and microenvironmental determinants of therapeutic response (Project 3).
CG, 9/09

Bizarri Receives Young Author Award

Greg Bizarri, a physicist in the Life Sciences Division, has been awarded the Young Author Award by the American Association for Crystal Growth. The Association noted that his "outstanding accomplishments in this field were decisive in the committee's opinion when choosing the winner. The award recognizes his contribution to the crystal growth community."

Greg Bizarri

The Young Author Award is presented to young persons (under 35 years of age) who have shown outstanding achievement in the field of crystal growth/epitaxy/modeling primarily through published papers. The first award was presented in 1981; subsequent awards have been presented at the Association's national meetings. Bizarri accepted his award at the 17th American Conference on Crystal Growth and Epitaxy - Lake Geneva, Wisconsin in August (http://www.crystalgrowth.us/accge17/index.php).

His awarded publication was on "Characterization and study of scintillator crystals: modeling and understanding of scintillation mechanisms". This publication discusses the basic concepts of scintillation in inorganic materials, focusing on how physics can provide pathways to assist in scintillator discovery. Finding new inorganic scintillator crystals with better performance for demanding applications, such as high-energy physics, medical imaging, and radiation detection, has been a long-standing challenge. Thanks to an intensive interdisciplinary effort between theoreticians, experimentalists and crystal growers, current scintillator performances are reaching the intrinsic limits

imposed by the crystal. However, demand continues for more efficient scintillators with higher light output, better energy resolution and lower cost of production.
Today at Berkeley Lab/CG, 9/29/09

Recent publications (selected)

Debolt S, Scheible WR, Schrick K, **Auer M**, Beisson F, Bischoff V, Bouvier-Nave P, Carroll A, Hematy K, Li Y, Milne J, Nair M, Schaller H, Zemla M, Somerville C. Mutations in UDP-glucose:sterol-glucosyltransferase in Arabidopsis cause transparent testa phenotype and suberization defect in seeds. *Plant Physiology*, 2009 Jul 29. [Epub ahead of print]PMID: 19641030

> In higher plants, the most abundant sterol derivatives are steryl glycosides and acyl steryl glycosides. Arabidopsis contains two genes, UGT80A2 and UGT80B1, that encode UDP-glucose:sterol glycosyltransferases, enzymes that catalyze the synthesis of steryl glycosides. Lines having mutations in UGT80A2, UGT80B1, or both UGT80A2 and UGT8B1 were identified and characterized. The ugt80A2 lines were viable and exhibited relatively minor effects on plant growth. Conversely, ugt80B1 mutants displayed an array of phenotypes that were pronounced in the embryo and seed. Most notable was the finding that ugt80B1 was allelic to tt15 and displayed a transparent testa phenotype and a reduction in seed size. In addition to the role of UGT80B1 in the deposition of flavanoids, a loss of suberization of the seed was apparent in ugt80B1 by the lack of autofluorescence at the hilum region. Moreover, in ugt80B1, scanning and transmission electron microscopy reveals that the outer integument of the seed coat lost the electron dense cuticle layer at its surface and displayed altered cell morphology. Gas chromatography coupled with mass spectrometry of lipid polyester monomers confirmed a drastic decrease in aliphatic suberin and cutin-like polymers that was associated with an inability to limit tetrazolium salt uptake. The findings suggest a membrane function for steryl glycosides and acyl steryl glycosides in trafficking of lipid polyester precursors. An ancillary observation was that cellulose biosynthesis was unaffected in the double mutant, inconsistent with a predicted role for steryl glycosides in priming cellulose synthesis.

Baran R, **Reindl W**, **Northen TR.** Mass spectrometry based metabolomics and enzymatic assays for functional genomics. *Current Opinion in Microbiology*, 2009 Aug 18. [Epub ahead of print] PMID: 19695948

> The exponential growth in the number of sequenced microorganisms versus the relative slow influx of direct biochemical characterization of microbes is limiting the utility of sequence information. High-throughput experimental approaches to functionally characterize microbial metabolism are urgently needed to leverage genome sequences for example: to understand host-microbe interactions, microbial communities, to utilize microbes for bioenergy, bioremediation, etc. Mass spectrometry based small molecule metabolite analysis is rapidly becoming a method of choice to meet these needs and enables multiple paths to discovering and validating the functional assignments. Approaches range from the targeted in vitro screening of small sets of metabolic transformations to define enzymatic activities to global metabolic profiling (metabolomics) to define metabolic pathways and gain insights into microbial responses to environmental and genetic perturbations. The combination of metabolite profiling with genome-scale models of metabolism and other -omic approaches provides opportunities to expand our understanding of microbial metabolic networks, stress responses, and to identify genes associated with specific enzymatic and regulatory activities.

Blakely EA, **Chang PY**. Biology of charged particles. *Cancer Journal*, 2009 Jul-Aug;15(4):271-84. PMID: 19672143

> Charged particles have moved from the physics laboratory to the clinic because of their advantageous dose profile and biologic effects. This brief review will summarize the basic phenomenological laboratory data that led to the successful clinical use of these modalities in selected tumor sites, and the emerging new genomic and proteomic research that have provided translational evidence for potential molecular mechanisms underlying some impressive clinical results.

MacArthur S, Li XY, Li J, **Brown JB**, Chu HC, **Zeng L**, Grondona BP, **Hechmer A**, **Simirenko L**, Keränen SV, **Knowles DW**, Stapleton M, Bickel P, **Biggin MD**, **Eisen MB**. Developmental roles of 21 Drosophila transcription factors are determined by quantitative differences in binding to an overlapping set of thousands of genomic regions. *Genome Biology*, 2009;10(7):R80. Epub 2009 Jul 23. PMID: 19627575

> BACKGROUND: We previously established that six sequence-specific transcription factors that initiate anterior/posterior patterning in Drosophila bind to overlapping sets of thousands of genomic regions in blastoderm embryos. While regions bound at high levels include known and probable functional targets, more poorly bound regions are preferentially associated with housekeeping genes and/or genes not transcribed in the blastoderm, and are frequently found in protein coding sequences or in less conserved non-coding DNA, suggesting that many are likely non-functional. RESULTS: Here we show that an additional 15 transcription factors that regulate other aspects of embryo patterning show a similar quantitative continuum of function and binding to thousands of genomic regions in vivo. Collectively, the 21 regulators show a surprisingly high overlap in the regions they bind given that they belong to 11 DNA binding domain families, specify distinct developmental fates, and can act via different cis-regulatory modules. We demonstrate, however, that quantitative differences in relative levels of binding to shared targets correlate with the known biological and transcriptional regulatory specificities of these factors. CONCLUSIONS: It is likely that the overlap in binding of biochemically and functionally unrelated transcription factors arises from the high concentrations of these proteins in nuclei, which, coupled with their broad DNA binding specificities, directs them to regions of open chromatin. We suggest that most animal transcription factors will be found to show a similar broad overlapping pattern of binding in vivo, with specificity achieved by modulating the amount, rather than the identity, of bound factor.

Campisi J, **Yaswen P**. Aging and cancer cell biology, 2009. *Aging Cell*, 2009 Jun;8(3):221-5. Epub 2009 Mar 27.PMID: 19627264

> Cancer is an age-related disease in organisms with renewable tissues. A malignant tumor arises in part from genomic damage, which can also drive age-related degeneration. However, cancer differs from many age-related degenerative diseases in that it entails gain-of-function changes that confer new (albeit aberrant) properties on cells, resulting in vigorous cell proliferation and survival. Nonetheless, interventions that delay age-related degeneration - for example, caloric restriction or dampened insulin/IGF-1 signaling - often also delay cancer. How then is the development of cancer linked to aging? The answer to this question is complex, as suggested by recent findings. This Hot Topic review discusses some of these findings, including how genomic damage might alter cellular properties without conferring mutations, and how some genes that regulate lifespan in organisms that lack renewable tissues might affect the development of cancer in mammals.

Phillips CM, McDonald KL, **Dernburg AF**. Cytological Analysis of Meiosis in Caenorhabditis elegans. *Methods Molecular Biology*. 2009;558:171-95. PMID: 19685325

The nematode Caenorhabditis elegans has emerged as an informative experimental system for analysis of meiosis, in large part because of the advantageous physical organization of meiotic nuclei as a gradient of stages within the germline. Here we provide tools for detailed observational studies of cells within the worm gonad, including techniques for light and electron microscopy.

Han B- G, Dong M, Liu H, Camp L, Geller J, Singer M, Hazen TC, Choi M, Witkowska HE, **Ball DA**, **Typke D**, **Downing KH**, Shatsky M, Brenner SE, Chandonia JM, **Biggin MD**, **Glaeser RM**. Survey of large protein complexes in *D. vulgaris* reveals unexpected structural diversity. *Proceedings of the National Academy of Sciences USA*. 2009

See GTL-Genomics Highlight, page 2.

Chen A, Cuevas I, **Kenny PA**, Miyake H, Mace K, **Ghajar C**, **Boudreau A**, **Bissell M**, Boudreau N. Endothelial cell migration and vascular endothelial growth factor expression are the result of loss of breast tissue polarity. *Cancer Research*, 2009 Aug 15;69(16):6721-9. [Epub 2009 Aug 4] PMID: 19654314

Recruiting a new blood supply is a rate-limiting step in tumor progression. In a three-dimensional model of breast carcinogenesis, disorganized, proliferative transformed breast epithelial cells express significantly higher expression of angiogenic genes compared with their polarized, growth-arrested nonmalignant counterparts. Elevated vascular endothelial growth factor (VEGF) secretion by malignant cells enhanced recruitment of endothelial cells (EC) in heterotypic cocultures. Significantly, phenotypic reversion of malignant cells via reexpression of HoxD10, which is lost in malignant progression, significantly attenuated VEGF expression in a hypoxia-inducible factor 1alpha-independent fashion and reduced EC migration. This was due primarily to restoring polarity: forced proliferation of polarized, nonmalignant cells did not induce VEGF expression and EC recruitment, whereas disrupting the architecture of growth-arrested, reverted cells did. These data show that disrupting cytostructure activates the angiogenic switch even in the absence of proliferation and/or hypoxia and restoring organization of malignant clusters reduces VEGF expression and EC activation to levels found in quiescent nonmalignant epithelium. These data confirm the importance of tissue architecture and polarity in malignant progression.

Coffey GP, Rajapaksa R, Liu R, Sharpe O, Kuo CC, **Krauss SW**, Sagi Y, Davis RE, Staudt LM, Sharman JP, Robinson WH, Levy S. Engagement of CD81 induces ezrin tyrosine phosphorylation and its cellular redistribution with filamentous actin. *Journal of Cell Science*, 2009 Aug 4. [Epub ahead of print] PMID: 19654214

CD81 is a tetraspanin family member involved in diverse cellular interactions in the immune and nervous systems and in cell fusion events. However, the mechanism of action of CD81 and of other tetraspanins has not been defined. We reasoned that identifying signaling molecules downstream of CD81 would provide mechanistic clues. We engaged CD81 on the surface of B-lymphocytes and identified the induced tyrosine-phosphorylated proteins by mass spectrometry. This analysis showed that the most prominent tyrosine phosphorylated protein was ezrin, an actin-binding protein and a member of the ezrin-radixin-moesin family. We also found that CD81 engagement induces spleen tyrosine kinase (Syk) and that Syk was involved in tyrosine phosphorylation of ezrin. After engagement of CD81, it colocalized with ezrin and F-actin, and this association was disrupted when Syk activation was blocked. Taken together, these studies suggest a model in which CD81 interfaces between the plasma membrane and the cytoskeleton by activating Syk, mobilizing ezrin, and recruiting F-actin to facilitate cytoskeletal reorganization and cell signaling. This mechanism might explain the pleiotropic effects induced in response to stimulation of cells by anti-CD81 antibodies or by the hepatitis C virus, which uses this molecule as its key receptor.

Landau SM, Harvey D, **Madison CM**, Koeppe RA, Reiman EM, Foster NL, Weiner MW, **Jagust WJ**; the Alzheimer's Disease Neuroimaging Initiative. Associations between cognitive, functional, and FDG-PET measures of decline in AD and MCI. *Neurobiology of Aging*, 2009 Aug 4. [Epub ahead of print] PMID: 19660834

> The Functional Activities Questionnaire (FAQ) and Alzheimer's Disease Assessment Scale-cognitive subscale (ADAS-cog) are frequently used indices of cognitive decline in Alzheimer's disease (AD). The goal of this study was to compare FDG-PET and clinical measurements in a large sample of elderly subjects with memory disturbance. We examined relationships between glucose metabolism in FDG-PET regions of interest (FDG-ROIs), and ADAS-cog and FAQ scores in AD and mild cognitive impairment (MCI) patients enrolled in the Alzheimer's Disease Neuroimaging Initiative (ADNI). Low glucose metabolism at baseline predicted subsequent ADAS-cog and FAQ decline. In addition, longitudinal glucose metabolism decline was associated with concurrent ADAS-cog and FAQ decline. Finally, a power analysis revealed that FDG-ROI values have greater statistical power than ADAS-cog to detect attenuation of cognitive decline in AD and MCI patients. Glucose metabolism is a sensitive measure of change in cognition and functional ability in AD and MCI, and has value in predicting future cognitive decline.

Liu Y, Prasad R, Beard WA, Hou EW, Horton JK, **McMurray CT**, Wilson SH. Coordination between Pol {beta} and FEN1 can modulate CAG repeat expansion. *The Journal of Biological Chemistry*, 2009 Aug 11. [Epub ahead of print] PMID: 19674974

> The oxidized DNA base 8-oxoguanine (8-oxoG) is implicated in neuronal CAG repeat expansion associated with Huntington's disease, yet it is unclear how such a DNA base lesion and its repair might cause the expansion. Here, we discovered size-limited expansion of CAG repeats during repair of 8-oxoG in a wild-type mouse cell extract. This expansion was deficient in extracts from cells lacking Pol beta and HMGB1. We demonstrate that expansion is mediated through Pol beta multi-nucleotide gap-filling DNA synthesis during long-patch base excision repair (BER). Unexpectedly, FEN1 promotes expansion by facilitating ligation of hairpins formed by strand slippage. This alternate role of FEN1 and the Pol beta multi-nucleotide gap-filling synthesis is the result of uncoupling of the usual coordination between Pol beta and FEN1. HMGB1 probably promotes expansion probably by stimulating APE1 and FEN1 in forming single-strand breaks and ligatable nicks, respectively. This is the first report illustrating that disruption of Pol beta and FEN1 coordination during long-patch BER results in CAG repeat expansion.

Pan D, Zhu Q, **Luo K**. SnoN functions as a tumour suppressor by inducing premature senescence. *The EMBO Journal*, 2009 Sep 10. [Epub ahead of print] PMID: 19745809

> SnoN represses TGF-beta signalling to promote cell proliferation and has been defined as a proto-oncogene partly due to its elevated expression in many human cancer cells. Although the anti-tumourigenic activity of SnoN has been suggested, the molecular basis for this has not been defined. We showed here that high levels of SnoN exert anti-oncogenic activity by inducing senescence. SnoN interacts with the promyelocytic leukaemia (PML) protein and is recruited to the PML nuclear bodies where it stabilizes p53, leading to premature senescence. Furthermore, overexpression of SnoN inhibits oncogenic transformation induced by Ras and Myc in vitro and significantly blocks papilloma development in vivo in a carcinogen-induced skin tumourigenesis model. The few papillomas that were developed displayed high levels of senescence and spontaneously regressed. Our study has revealed a novel Smad-independent pathway of SnoN function that mediates its anti-oncogenic activity.

Mori H, Gjorevski N, **Inman JL**, **Bissell MJ**, Nelson CM. Self-organization of engineered epithelial tubules by differential cellular motility. *Proceedings of the National Academy of Sciences, U S A*, 2009 Aug 18. [Epub ahead of print] PMID: 19706461

Patterning of developing tissues arises from a number of mechanisms, including cell shape change, cell proliferation, and cell sorting from differential cohesion or tension. Here, we reveal that differences in cell motility can also lead to cell sorting within tissues. Using mosaic engineered mammary epithelial tubules, we found that cells sorted depending on their expression level of the membrane-anchored collagenase matrix metalloproteinase (MMP)-14. These rearrangements were independent of the catalytic activity of MMP14 but absolutely required the hemopexin domain. We describe a signaling cascade downstream of MMP14 through Rho kinase that allows cells to sort within the model tissues. Cell speed and persistence time were enhanced by MMP14 expression, but only the latter motility parameter was required for sorting. These results indicate that differential directional persistence can give rise to patterns within model developing tissues.

Hancock JM, Mallon AM, Beck T, Gkoutos GV, **Mungall C**, Schofield PN. Mouse, man, and meaning: bridging the semantics of mouse phenotype and human disease. *Mammalian Genome*. 2009 Aug 2. [Epub ahead of print] PMID: 19649761

Now that the laboratory mouse genome is sequenced and the annotation of its gene content is improving, the next major challenge is the annotation of the phenotypic associations of mouse genes. This requires the development of systematic phenotyping pipelines that use standardized phenotyping procedures which allow comparison across laboratories. It also requires the development of a sophisticated informatics infrastructure for the description and interchange of phenotype data. Here we focus on the current state of the art in the description of data produced by systematic phenotyping approaches using ontologies, in particular, the EQ (Entity-Quality) approach, and what developments are required to facilitate the linking of phenotypic descriptions of mutant mice to human diseases.

Porter-Chapman YD, Bourret-Courchesne ED, **Bizarri GA, Weber MJ, Derenzo SE.** Scintillation and luminescence properties of undoped and cerium-doped $LiGdCl_4$ and $NaGdCl_4$. *IEEE Transactions on Nuclear Science* 56(3):881–886, June 2009.

We report the scintillation properties of the undoped and cerium-doped variations of $LiGdCl_4$ and $NaGdCl_4$. Powder samples of these materials exhibit significant scintillation under X-rays. The samples were synthesized by solid-state methods from a 1:1 molar ratio of lithium or sodium chloride and gadolinium trichloride. Cerium trichloride was used as the dopant. The physical, optical, and scintillation properties of these materials were analyzed by powder X-ray diffraction, photoluminescence, X-ray excited luminescence, and pulsed X-ray luminosity measurements. Increases in light yields are observed as the concentration of cerium increases. The highest light yields occurred at 20% cerium doping for both compounds. At larger concentrations neither compound formed, indicating a breakdown of the lattice with the addition of large amounts of cerium cations. At 20% cerium, $LiGdCl_4$ and $NaGdCl_4$ display scintillation light 3.6 times and 2.2 times the light yield of the reference material, $YAlO_3 : Ce^{3+}$, respectively. Both emit in the ranges of 340–350 nm and 365–370 nm and display multiexponential decays with cerium-like decay components at 33 ns ($LiGdCl_4 : Ce$) and 26 ns ($NaGdCl_4 : Ce$).

Campeau E, Ruhl VE, **Rodier F**, Smith CL, Rahmberg BL, **Fuss JO**, **Campisi J**, **Yaswen P**, **Cooper PK**, Kaufman PD. A versatile viral system for expression and depletion of proteins in mammalian cells. *PLoS One.* 2009 Aug 6;4(8):e6529. PMID: 19657394

The ability to express or deplete proteins in living cells is crucial for the study of biological processes. Viral vectors are often useful to deliver DNA constructs to cells that are difficult to transfect by other methods. Lentiviruses have the additional advantage of being able to integrate into the genomes of non-dividing mammalian cells. However, existing viral expression systems generally require different vector backbones

for expression of cDNA, small hairpin RNA (shRNA) or microRNA (miRNA) and provide limited drug selection markers. Furthermore, viral backbones are often recombinogenic in bacteria, complicating the generation and maintenance of desired clones. Here, we describe a collection of 59 vectors that comprise an integrated system for constitutive or inducible expression of cDNAs, shRNAs or miRNAs, and use a wide variety of drug selection markers. These vectors are based on the Gateway technology (Invitrogen) whereby the cDNA, shRNA or miRNA of interest is cloned into an Entry vector and then recombined into a Destination vector that carries the chosen viral backbone and drug selection marker. This recombination reaction generates the desired product with >95% efficiency and greatly reduces the frequency of unwanted recombination in bacteria. We generated Destination vectors for the production of both retroviruses and lentiviruses. Further, we characterized each vector for its viral titer production as well as its efficiency in expressing or depleting proteins of interest. We also generated multiple types of vectors for the production of fusion proteins and confirmed expression of each. We demonstrated the utility of these vectors in a variety of functional studies. First, we show that the FKBP12 Destabilization Domain system can be used to either express or deplete the protein of interest in mitotically-arrested cells. Also, we generate primary fibroblasts that can be induced to senesce in the presence or absence of DNA damage. Finally, we determined that both isoforms of the AT-Rich Interacting Domain 4B (ARID4B) protein could induce G1 arrest when overexpressed. As new technologies emerge, the vectors in this collection can be easily modified and adapted without the need for extensive recloning.

Sarkar P, **Bosneaga E, Auer M**. Plant cell walls throughout evolution: towards a molecular understanding of their design principles. *Journal of Experimental Botany*, 2009 Aug 17. [Epub ahead of print] PMID: 19687127

Throughout their life, plants typically remain in one location utilizing sunlight for the synthesis of carbohydrates, which serve as their sole source of energy as well as building blocks of a protective extracellular matrix, called the cell wall. During the course of evolution, plants have repeatedly adapted to their respective niche, which is reflected in the changes of their body plan and the specific design of cell walls. Cell walls not only changed throughout evolution but also are constantly remodelled and reconstructed during the development of an individual plant, and in response to environmental stress or pathogen attacks. Carbohydrate-rich cell walls display complex designs, which together with the presence of phenolic polymers constitutes a barrier for microbes, fungi, and animals. Throughout evolution microbes have co-evolved strategies for efficient breakdown of cell walls. Our current understanding of cell walls and their evolutionary changes are limited as our knowledge is mainly derived from biochemical and genetic studies, complemented by a few targeted yet very informative imaging studies. Comprehensive plant cell wall models will aid in the re-design of plant cell walls for the purpose of commercially viable lignocellulosic biofuel production as well as for the timber, textile, and paper industries. Such knowledge will also be of great interest in the context of agriculture and to plant biologists in general. It is expected that detailed plant cell wall models will require integrated correlative multimodal, multiscale imaging and modelling approaches, which are currently underway.

Weigelt B, Lo AT, **Park CC**, **Gray JW**, **Bissell MJ**. HER2 signaling pathway activation and response of breast cancer cells to HER2-targeting agents is dependent strongly on the 3D microenvironment. *Breast Cancer Research and Treatment*, 2009 Aug 22. [Epub ahead of print] PMID: 19701706

Development of effective and durable breast cancer treatment strategies requires a mechanistic understanding of the influence of the microenvironment on response. Previous work has shown that cellular signaling pathways and cell morphology are dramatically influenced by three-dimensional (3D) cultures as opposed to traditional two-dimensional (2D) monolayers. Here, we compared 2D and 3D culture models to determine the impact of 3D architecture and extracellular matrix (ECM) on HER2 signaling and on the response of HER2-amplified breast cancer cell lines to the HER2-targeting agents

Trastuzumab, Pertuzumab and Lapatinib. We show that the response of the HER2-amplified AU565, SKBR3 and HCC1569 cells to these anti-HER2 agents was highly dependent on whether the cells were cultured in 2D monolayer or 3D laminin-rich ECM gels. Inhibition of beta1 integrin, a major cell-ECM receptor subunit, significantly increased the sensitivity of the HER2-amplified breast cancer cell lines to the humanized monoclonal antibodies Trastuzumab and Pertuzumab when grown in a 3D environment. Finally, in the absence of inhibitors, 3D cultures had substantial impact on HER2 downstream signaling and induced a switch between PI3K-AKT- and RAS-MAPK-pathway activation in all cell lines studied, including cells lacking HER2 amplification and overexpression. Our data provide direct evidence that breast cancer cells are able to rapidly adapt to different environments and signaling cues by activating alternative pathways that regulate proliferation and cell survival, events that may play a significant role in the acquisition of resistance to targeted therapies.

Williams PT. Relationship of incident glaucoma versus physical activity and fitness in male runners. *Medicine and Science in Sports and Exercise*, 2009 Aug;41(8):1566-72. PMID: 19568204

PURPOSE: To assess the dose-response relationship of vigorous physical activity (running distance, km x d(-1)) or cardiorespiratory fitness (meters-per-second pace during a 10-km footrace) to the risk for incident glaucoma. DESIGN: Prospective epidemiologic cohort study. METHODS: Participant-reported, physician-diagnosed incident glaucoma was compared with distance run per week and 10-km footrace performance in a cohort of 29,854 male runners without diabetes followed prospectively for 7.7 yr. The survival analyses were adjusted for age, hypertension, current and past cigarette use, and intakes of meat, fish, fruit, and alcohol. RESULTS: Two hundred incident glaucoma cases were reported during follow-up. The risk for reported glaucoma decreased 37% per meter per second increment in a 10-km race performance (P = 0.005). Relative to the least fit men (i.e., slowest, < or = 3.5 m x s(-1)), the risk for incident-reported glaucoma declined 29% in those who ran 3.6-4.0 m x s(-1) (P = 0.06), 54% for those who ran 4.1-4.5 m x s(-1) (P = 0.001), 51% for those who ran 4.6-5.0 m x s(-1) (P = 0.04), and glaucoma was nonexistent among the 781 men who exceeded 5.0 m x s(-1) (P = 0.03). The risk for incident, reported glaucoma decreased 5% per kilometer per day run at baseline (P = 0.04), which remained significant when adjusted for the 10-km race performance (5% reduction per kilometer per day, P = 0.04), and both body mass index and race performance (P = 0.04). Baseline hypertension was unrelated to the incident glaucoma. CONCLUSIONS: These data provide preliminary evidence that vigorous physical activity may reduce glaucoma risk, which, in the absence of medical record validation, could represent ocular hypertension in addition to frank glaucoma. Additional follow-up with validation is needed to identify the type of glaucoma affected.